DEFICIENCY IN INITIATION FACTORS OF PROTEIN SYNTHESIS INDUCED BY PHAGE T7 IN *E. COLI* F^+ STRAINS

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1. Introduction

The fate of *E. coli* bacteriophage T7 (and related phages) is determined by the sex of the host: female cells support complete T7 development resulting in a rapid release of infective particles, while T7 phage maturation is abortive in male (F^+ or F') cells. Linial and Malamy [1] have shown that this lack of T7 growth in male hosts is under translational control, all the T7 mRNA detected in females being also found in F^+ cells. Morrison and Malamy [2] recently reported that following infection of *E. coli* F^+ cells by phage T7, the early T7 genes are translated, but no late proteins are made; moreover, crude cell-free extracts from T7 infected male cells have no protein synthesis activity [3].

We have used immunological techniques to follow the amount of initiation factors, in particular IF3, in extracts of F^+ and F^- *E. coli* strains, before and after infection by phages T7 and T4.

Upon infection of F^+ strains with phage T7, we have observed a dramatic and rapid loss of initiation factor IF3. This does not occur with phage T4 or in F^- *E. coli* strains. This observation could account for the block of late T7 mRNA translation in male strains reported by Morrison and Malamy [2].

2. Materials and methods

For most of these studies, we used the isogenic pair of *E. coli* strains RV and RVF′ kindly supplied by Dr. M. Malamy. T7 was obtained from Dr. Nygaardt. Bacteria, grown in B broth (containing per liter 10 g bactotryptone, 5 g NaCl) at 30° to an absorbance of 0.4 at 600 nm, were infected with T7 (MOI = 10–15). The T7 infected cells were harvested after 13 min and washed once in buffer A (0.01 M Tris-HCl pH 7.5, 0.06 M NH_4Cl, 0.01 M $MgCl_2$, 7 mM β-mercapto-ethanol). T4-infected cells were grown and harvested according to Salser et al. [4]. The fractionation of *E. coli* extracts and the preparation of crude initiation factors (2 M NH_4Cl ribosomal wash proteins) has been described in detail elsewhere [5]. Purification of IF3 and interference factor *i* and the preparation of rabbit antiserum were reported previously [6,7]. Quantitative estimates of the amount of IF3 and factor *i* antigens present within the ribosomal wash protein mixtures were obtained by microcomplement fixation [8] as detailed in the legends of the figures. The activity of crude initiation factors was measured by poly (AUG) dependent formyl-[^{35}S] methionyl-tRNA binding and natural mRNA directed amino acid incorporation as before [5].

3. Results

Fig. 1a shows that in the ribosomal wash proteins of *E. coli* RVF′ infected with T7 there is an almost complete loss of IF3 antigen. In a series of experiments the decrease in IF3 averaged 90% after 13 min of infection at 30°.

In contrast, no IF3 decrease was detected after T7 infection of the isogenic RV strain (fig. 1c). A sharpening of the complement fixation curve was nevertheless observed, which could indicate a liberation of IF3 from an aggregated form (D. Stollar, personal communication).

Other F^+ strains like *E. coli* 58–161 underwent a

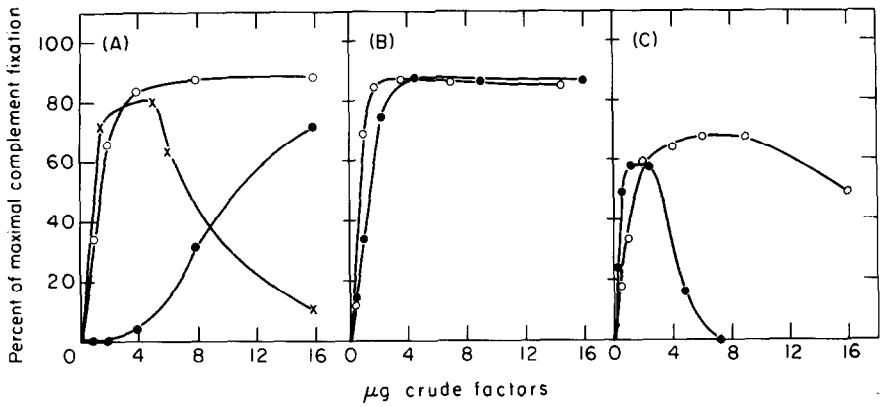

Fig. 1. Quantitative estimates by complement (C′) fixation of initiation factor IF3 before and after infection with bacteriophage T7 or T4. The reaction mixture contained in 1.2 ml, 0.2 ml of guinea-pig C′ diluted 1/175, anti IF3 purified as in [16] and ribosomal wash proteins (crude factors) as indicated, to which were added after an overnight incubation at 0°, 0.2 ml of sheep erythrocytes sensitized with hemolysin 1/400. Hemolysis by unbound C′ was allowed to occur at 37° for 40 min. The extent of C′ fixation was estimated as precedently described [5]. Protein concentrations were determined by the method of Lowry [17].

(A) Effect of T7 infection on *E. coli* RVF′ cells. The above reaction mixture contained in addition 9 μg anti IF3. Ribosomal wash proteins were from (o–o–o) noninfected RVF′; (•–•–•) T7 infected RVF′; (X–X–X) T7 infected RVF′ treated with chloramphenicol (200 μg/ml) 5 min prior to infection.

(B) Effect of T4 infection on *E. coli* RVF′ cells. The reaction mixture included 9 μg anti IF3. Ribosomal wash proteins were from (o–o–o) noninfected RVF′; (•–•–•) T4 infected RVF′.

(C) Effect of T7 infection on *E. coli* RV cells. The reaction mixture contained 7 μg anti IF3. Ribosomal wash proteins were from (o–o–o) noninfected RV; (•–•–•) T4 infected RV.

similar loss in IF3 after T7 infection. The IF3 antigenic activity which disappears from the ribosome associated fraction does not appear in the supernatant soluble fraction of the extracts.

The loss of IF3 is also seen when biological activity is measured. Very little initiation factor activity for the translation of T7 mRNA was found in the ribosomal wash proteins from T7 infected *E. coli* RVF′ cells (table 1b). This low activity is not due to the presence of an inhibitor. There is, however, a decrease also in IF2 activity (table 1a). This decrease, which amounted to less than 50% was, nevertheless, much less dramatic than that in IF3. The biological activity of the crude initiation factors from *E. coli* RV was not significantly changed after T7 infection.

The loss of IF3 after T7 infection of *E. coli* RVF′ requires protein synthesis since it is blocked by the addition of chloramphenicol 5 min prior to phage infection (fig. 1a). The effect appears very early after T7 infection: as shown in fig. 2, it is already marked at 1 min and levels off at about 5 min after infection at 30°.

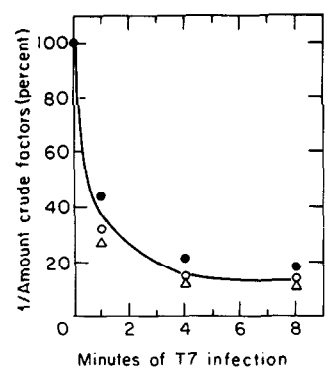

Fig. 2. Time curve of the disappearance of IF3 after T7 infection of *E. coli* RVF′. The amount of IF3 antigen was determined quantitatively as described in fig. 1 in ribosomal wash proteins (crude factors) from *E. coli* RVF′ at different times after T7 infection. The inverse amounts of crude factors needed to give a 30 (•–•–•), 50 (o–o–o) or 70 (△–△–△) percent complement fixation were used as three measures of the concentration of IF3 antigen. Results are expressed in per cent of the non-infected extract.

Table 1
Initiation factor activity in extracts from *E. coli* RVF'.

1a. Expt. 1:	Assay of IF2 activity	
Ribosomal wash proteins added:	Poly AUG dependent [S^{35}] fmet tRNA binding (cpm)	Per cent of control
Normal, 15 μg	12,320	100
Normal, 30 μg	18,525	100
T7 infected, 16 μg	6,540	53
T7 infected, 32 μg	10,590	53
1b. Expt. 2:	Assay of IF1, IF2, IF3 activity	
Ribosomal wash proteins added:	T7 mRNA dependent [C^{14}] valine incorporation (cpm)	Per cent of control
Normal, 27 μg	6,300	100
Normal, 54 μg	12,400	100
T7 infected, 32 μg	1,085	14
T7 infected, 65 μg	2,380	15

Poly AUG dependent [^{35}S] fmet-tRNA (2000 μCi/μmole) binding to 200 μg ribosomes was measured in the presence of purified IF1 and IF3 as described elsewhere [5,7]. A background without added IF2 of 1,850 cpm was subtracted. T7 mRNA (prepared as in [4] from *E. coli* B, 13 min after T7 infection at 30°) dependent [^{14}C] valine (50 μCi/μmole) incorporation was measured with 160 μg ribosomes as before [5]. A background without factors of 1380 cpm was subtracted.

The decrease in IF3 in the F^+ strain is clearly bacteriophage specific: fig. 1b shows that infection of *E. coli* RVF' by phage T4 does not produce any change in the amount of IF3 present. T4 infection had no effect on the *amount* of IF3 measured by immunological techniques, in a number of *E. coli* strains, although it produces a change in the template specificity of IF3 activity [9].

Changes in template specificity could be due to interference factors [6]. We have therefore also followed by similar immunological techniques the fate of interference factor *i*. This protein is a cistron specific initiation factor which inhibits the translation of certain cistrons like the coat protein cistron of MS2 RNA, while promoting that of other cistrons such as the MS2 synthetase [10]. It also stimulates the translation of T7 mRNA's (H. Zeller and M. Revel, unpublished.

A small decrease in factor *i* was occasionally observed in extracts from T7 infected RVF' (fig. 3). This drop was never more than twofold, much less therefore than that affecting IF3.

4. Discussion

The mechanism by which IF3 is lost after T7 infection in F^+ strains of *E. coli* is unknown. It probably involves a degradation of the protein itself, since our preliminary results indicate that it is abolished by treatment with the protease inhibitor phenylmethanesulphonyl fluoride [11]. A deficiency in IF3, although less dramatic, has been observed before in stationary phase *E. coli* [5] and in *E. coli* treated with chloramphenicol for long periods of time [12]. This decrease does not result only from the arrest of protein synthesis since amino acid starvation does not cause a drop in IF3 [13]. Moreover, after T4 infection, although there is a complete shut-off of host RNA and protein synthesis, no decrease in the amount of IF3 is observed. This suggests a rather specific mechanism for the regulation of the level of IF3 in the cell.

The loss of IF3 may be responsible for the effects on T7 development in *E. coli* RVF' observed by Morrison and Malamy [2]. This loss would stop protein synthesis and since it occurs early after T7 in-

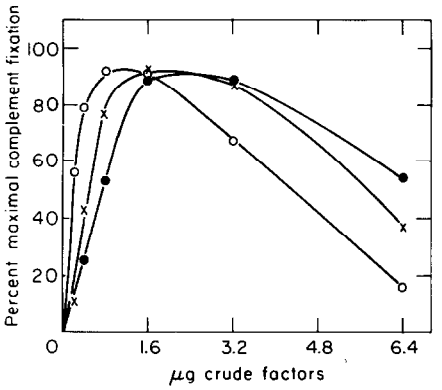

Fig. 3. Quantitative immunoassay of interference factor *i* in RVF' cells before and after infection by T7. The C'fixation assay was performed as described in fig. 1 and contained 0.8 μg of purified anti *i* kindly provided by Dr. Y. Groner. Ribosomal wash proteins (crude factors) were from (o–o–o) noninfected RVF'; (•–•–•) T7 infected RVF'; (X–X–X) chloramphenicol-treated T7 infected RVF', as in fig. 1.

fection, only some early gene products are made, the late mRNA's not being translated any more. If this is the case, it is of great interest that some episomal mutants in the F factor can make at least some late T7 proteins: this might result from a delay in the loss of IF3.

A role of interference factors [6] in the control of T7 mRNA translation is also possible. We have detected, at present, two interference factors active on T7 mRNA translation: factor *i* stimulates this translation, while the other factor inhibits T7 mRNA translation (H. Zeller and M. Revel, unpublished). The effects of both factors compete with that of IF3 itself. Any change in one of these components can alter the mRNA specificity of ribosomes.

Phage infection appears to alter the balance between initiation factors of protein synthesis. In this connection, it is interesting to mention that after T4 infection, a modification of interference factor *i* is observed (Y. Pollack, R. Scheps, H. Inouye and M. Revel, unpublished results). This modification, which is detected by an increased mobility of the protein toward the anode upon immunoelectrophoresis at pH 8.8, is an early event after T4 infection. This observation, the known changes in initiation factor template specificity after T4 infection [9, 14, 15] and the effects on IF3 described in the present article suggest that viruses interfere with the normal mechanism of protein synthesis, not only by substituting their own mRNA's to the cellular ones, but also by altering the machinery for mRNA translation.

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